

**WHAT IS CLAIMED IS:**

1. A method for sequencing a target nucleic acid, comprising the steps of:

providing a set of nucleic acid fragments each containing a sequence that corresponds to a sequence of the target nucleic acid;

5 hybridizing the set to an array of nucleic acid probes to form a target array of nucleic acids, wherein each probe comprises a single-stranded portion comprising a variable region; and

determining molecular weights for nucleic acids of the target array;

10 whereby the sequence of the target nucleic acid is determined.

2. The method of claim 1, wherein the molecular weights are determined by methods selected from the group consisting of gel electrophoresis, capillary electrophoresis, chromatography, and nuclear magnetic resonance.

15 3. The method of claim 1, wherein the molecular weights are determined by mass spectrometry.

4. The method of claim 3, wherein the mass spectrometry comprises a step selected from the group consisting of laser heating, droplet release, electrical release, photochemical release, fast atom

20 bombardment, plasma desorption, matrix-assisted laser desorption/ionization, electrospray, and resonance ionization, or a combination thereof.

5. The method of claim 3, wherein the mass spectrometry comprises a step selected from the group consisting of Fourier Transform, ion cyclotron resonance, time of flight analysis with reflection, time of

25 flight analysis without reflection, and quadrupole analysis, or a combination thereof.

6. The method of claim 3, wherein the mass spectrometry comprises matrix-assisted desorption ionization and time of flight analysis.

30 7. The method of claim 3, wherein the mass spectrometry comprises electrospray ionization and quadrupole analysis.

8. The method of claim 3, wherein two or more molecular weights are determined simultaneously.

9. The method of claim 1, further comprising the step of enzymatically extending the nucleic acid probes of the target array using 5 the hybridized target nucleic acid as a template to form extended strands.

10. The method of claim 2, wherein the extended strands comprise DNA, RNA, PNA or combinations thereof.

11. The method of claim 9, wherein the step of extending is performed in the presence of chain elongating nucleotides and chain 10 terminating nucleotides.

12. The method of claim 1, wherein the array comprises nucleic acid probes having at least one mass-modifying functionality.

13. The method of claim 12, wherein the mass-modifying functionality is coupled to a heterocyclic base, a sugar moiety or a 15 phosphate group.

14. The method of claim 12, wherein the mass-modifying functionality is a chemical moiety that does not interfere with hydrogen bonding for base-pair formation.

15. The method of claim 12, wherein the mass-modifying 20 functionality is coupled to a purine at position C2, N3, N7, or C8.

16. The method of claim 12, wherein the mass-modifying functionality is coupled to a deazapurine at position N7 or N9.

17. The method of claim 12, wherein the mass-modifying functionality is coupled to a pyrimidine at position C5 or C6.

25 18. The method of claim 12, wherein the mass-modifying functionality is selected from the group consisting of F, Cl, Br, I, SiR<sub>3</sub>, Si(CH<sub>3</sub>)<sub>3</sub>, Si(CH<sub>3</sub>)<sub>2</sub>(C<sub>2</sub>H<sub>5</sub>), Si(CH<sub>3</sub>)(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>, Si(C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>, (CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>, (CH<sub>2</sub>)<sub>n</sub>NR<sub>2</sub>, CH<sub>2</sub>CONR<sub>2</sub>, (CH<sub>2</sub>)<sub>n</sub>OH, CH<sub>2</sub>F, CHF<sub>2</sub>, and CF<sub>3</sub>;

wherein n is an integer; and

30 wherein R is selected from the group consisting of -H, deuterium and alkyls, alkoxy and aryls of 1-6 carbon atoms, polyoxymethylene,

monoalkylated polyoxymethylene, polyethylene imine, polyamide, polyester, alkylated silyl, heterooligo/polyaminoacid and polyethylene glycol.

19. The method of claim 12, wherein the mass-modifying functionality is  $-N_3$  or  $-XR$ ,  
5 wherein X is selected from the group consisting of  $-O-$ ,  $-NH-$ ,  $-NR-$ ,  
 $-S-$ ,  $-OCO(CH_2)_nCOO-$ ,  $-NHCO(CH_2)_nCOO-$ ,  $-OSO_2O-$ ,  $-OCO(CH_2)_n-$ ,  
 $-NHC(O)-$ , and  $-C(O)NH-$ , and n is an integer from 1 to 20; and  
10 wherein R is selected from the group consisting of  $-H$ , deuterium  
and alkyls, alkoxy and aryls of 1-6 carbon atoms, polyoxymethylene,  
monoalkylated polyoxymethylene, polyethylene imine, polyamide,  
polyester, alkylated silyl, heterooligo/polyaminoacid and polyethylene  
glycol.

20. The method of claim 19, wherein X is  $-NHC(S)-$ .  
15 21. The method of claim 19, wherein X is  $-NHC(S)NH-$ .  
22. The method of claim 19, wherein X is  $-NC_4O_2H_3S-$ .  
23. The method of claim 19, wherein X is  $-OCO(CH_2)_nS-$ .  
24. The method of claim 23, wherein X is  $-OCO(CH_2)S-$ .  
25. The method of claim 19, wherein X is  $-OP(O-alkyl)-$ .  
20 26. The method of claim 19, wherein X is  $-OPO(O-alkyl)-$ .  
27. The method of claim 12, wherein the mass-modifying  
functionality is a thiol moiety.  
28. The method of claim 20, wherein the thiol moiety is  
generated by using Beu cage reagent.  
25 29. The method of claim 12, wherein the mass-modifying  
functionality is an alkyl moiety.  
30. The method of claim 29, wherein the alkyl moiety is  
generated by using iodoacetamide.  
31. The method of claim 1, further comprising the step of  
30 removing alkali cations.

32. The method of claim 24, wherein the alkali cations are removed by ion exchange.

33. The method of claim 32, wherein the ion exchange comprises contacting the nucleic acid with a solution selected from the group consisting of ammonium acetate, ammonium carbonate, diammonium hydrogen citrate, and ammonium tartrate, or combinations thereof.

34. The method of claim 1, further comprising the step of ligating the hybridized target nucleic acids to the probes.

10 35. The method of claim 1, wherein the target nucleic acid is provided from a biological sample.

36. The method of claim 35, wherein the biological sample is obtained from a patient.

15 37. The method of claim 1, wherein the target nucleic acid is provided from a recombinant source.

38. The method of claim 1, where the target nucleic acid is between about 10 to about 1,000 nucleotides in length.

39. The method of claim 1, where the nucleic acid fragments are between about 10 to about 1,000 nucleotides in length.

20 40. The method of claim 1, wherein each sequence of the nucleic acid fragments is homologous with at least a portion of the sequence of the target nucleic acid.

41. The method of claim 1, wherein each sequence of the set of nucleic acid fragments is complementary with at least a portion of the sequence of the target nucleic acid.

25 42. The method of claim 1, comprising the step of dephosphorylating the nucleic acid fragments by treatment with a phosphatase prior to hybridization.

43. The method of claim 1, wherein the fragments are provided by enzymatic digestion of the target nucleic acid.

30

44. The method of claim 36, wherein the enzymatic digestion is carried out by a nuclease.

45. The method of claim 1, wherein the nucleic acid fragments are provided by physically cleaving the target nucleic acid.

5 46. The method of claim 1, wherein the nucleic acid fragments are provided by enzymatic polymerization, wherein the target nucleic acid is a template.

10 47. The method of claim 46, wherein the enzymatic polymerization is a nucleic acid amplification process selected from the group consisting of strand displacement amplification, ligase chain reaction, Q $\beta$  replicase amplification, 3SR amplification, and polymerase chain reaction.

15 48. The method of claim 46, wherein the enzymatic polymerization is carried out in the presence of chain elongating nucleotides and chain terminating nucleotides.

49. The method of claim 1, wherein the nucleic acid fragments are provided by synthesizing a complementary copy of the target sequence.

20 50. The method of claim 1, wherein the nucleic acid fragments comprise a nested set.

51. The method of claim 1, wherein the nucleic acid fragments comprise DNA, RNA, PNA or combinations thereof.

52. The method of claim 1, wherein the target nucleic acid comprises DNA, RNA, PNA or modifications of combinations thereof.

25 53. The method of claim 1, wherein the fragments of nucleic acids comprise greater than about 10<sup>4</sup> different members and each member is between about 10 to about 1,000 nucleotides in length.

54. The method of claim 1, wherein the probes are single-stranded.

30 55. The method of claim 1, wherein the array comprises a collection of probes with sufficient sequence diversity in the variable

regions to hybridize all of the target sequence with complete or nearly complete discrimination.

56. The method of claim 1, wherein the probes comprise a double-stranded portion and a single-stranded portion.

5 57. The method of claim 56, wherein the probes have a single-stranded region at one terminus and a double-stranded region at the opposite terminus.

58. The method of claim 1, wherein the probes are about 10 to about 1,000 nucleotides in length.

10 59. The method of claim 1, wherein the probes are about 15 to about 200 nucleotides in length.

60. The method of claim 1, wherein the probes are about 10 to 50 nucleotides in length.

15 61. The method of claim 56, wherein the double-stranded portion is about 4 to about 30 nucleotides in length.

62. The method of claim 56, wherein the single-stranded portion is about 4 to about 20 nucleotides in length.

63. The method of claim 1, wherein the variable region is about 4 to about 20 nucleotides in length.

20 64. The method of claim 1, wherein the array of nucleic acid probes is attached to a solid support.

65. The method of claim 64, wherein the solid support is selected from the group consisting of plates, beads, microbeads, whiskers, combs, hybridization chips, membranes, single crystals, 25 ceramics, and self-assembling monolayers.

66. The method of claim 64, wherein the probes are conjugated with biotin or a biotin derivative and wherein the solid support is conjugated with avidin, streptavidin or a derivative thereof.

30 67. The method of claim 64, wherein each probe is attached to the solid support by a bond selected from the group consisting of covalent bond, electrostatic bond, hydrogen bond, cleavable bond,

*Sub C1*

*Subc1* → photocleavable bond, disulfide bond, peptide bond, diester bond, and selectively releasable bond, or a combination thereof.

68. The method of claim 67, wherein the cleavable bond is cleaved by a cleaving agent selected from the group consisting of heat, 5 an enzyme, a chemical agent, and electromagnetic radiation, or a combination thereof.

69. The method of claim 68, wherein the chemical agent is selected from the group consisting of reducing agents, oxidizing agents, and hydrolyzing agents, or a combination thereof.

10 70. The method of claim 68, wherein the electromagnetic radiation is selected from the group consisting of visible radiation, ultraviolet radiation, and infrared radiation.

71. The method of claim 67, wherein the selectively releasable bond is 4, 4'-dimethoxytrityl or a derivative thereof.

15 72. The method of claim 71, wherein the derivative is selected from the group consisting of 3 or 4 [bis-(4-methoxyphenyl)]-methylbenzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-methylbenzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-hydroxymethyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-

20 methoxyphenyl)]-chloromethyl-benzoic acid and salts thereof.

73. The method of claim 64, comprising a spacer between each probe and the solid support.

74. The method of claim 73, wherein the spacer is selected from the group consisting of oligopeptides, oligonucleotides, oligopolyamides, 25 oligoethyleneglycerol, oligoacrylamides, and alkyl chains of between about 6 to about 20 carbon atoms, or combinations thereof.

75. The method of claim 64, wherein the solid support comprises a matrix that facilitates volatilization of nucleic acids for molecular weight determination.

30 76. The method of claim 1, wherein the nucleic acid probes comprise DNA, RNA, PNA, or combinations thereof.

*Extr B1*

77. A method of detecting a target nucleic acid, comprising the steps of:

providing a set of nucleic acid fragments each containing a sequence that corresponds to a sequence of the target nucleic acid;

5 hybridizing the set to an array of nucleic acid probes to form a target array of nucleic acids, wherein each probe comprises a single-stranded portion comprising a variable region; and

determining molecular weights for nucleic acids of the target array; whereby the sequence of the target nucleic acid is determined.

10 78. An array of nucleic acid probes, wherein each probe comprises a single-stranded portion comprising a variable sequence, and

wherein the array is attached to a solid support comprising a matrix that facilitates the volatilization of nucleic acids for mass spectrometry.

15 79. The array of claim 78, wherein the array comprises a collection of probes with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete or nearly complete discrimination.

80. The array of claim 78, wherein the nucleic acid probes

20 comprise a single-stranded portion and a double-stranded portion.

81. The array of claim 78, wherein the array comprises nucleic acid probes having at least one mass-modifying functionality.

82. A kit for sequencing and detecting a target nucleic acid, comprising

25 an array of nucleic acid probes fixed to a solid support, wherein each probe comprises a single-stranded portion comprising a variable sequence, and

wherein the solid support comprises a matrix that facilitates volatilization of nucleic acids for mass spectrometry.

30 83. The kit of claim 82, wherein the array comprises a collection of probes with sufficient sequence diversity in the variable regions to

hybridize all of the target sequence with complete or nearly complete discrimination.

84. The kit of claim 82, wherein the nucleic acid probes comprise a single-stranded portion and a double-stranded portion.

5 85. A system for sequencing and detecting a target nucleic acid, comprising:

a mass spectrometer;

a computer; and

an array of nucleic acid probes,

10 wherein each probe comprises a single-stranded portion comprising a variable sequence, and

wherein the array of nucleic acid probes is attached to a solid support.

15 86. The system of claim 85, wherein the array comprises a collection of probes with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete diversity or nearly complete diversity.

87. The system of claim 85, wherein the nucleic acid probes comprise a single-stranded portion and a double-stranded portion.

Add a'

Add B4